3rd ISMB Retreat
24-25 June 2009

Report by Dr. Clare Sansom

The biennial Institute of Structural and Molecular Biology retreat, designed to build links and foster collaborations between scientists in the five departments of the Institute, was held for the third time in June 2009. Almost exactly two years earlier, the second retreat had been held at the Wellcome Trust Conference Centre on the Genome Campus at Hinxton, near Cambridge. In 2009 the Institute moved into the city centre, to the newest of the thirty-one Cambridge colleges. Robinson College was opened in 1981, largely funded by the entrepreneur and philanthropist, Sir David Robinson, whose name it bears. Its quiet, roomy red-brick buildings proved an ideal environment for two half-days of excellent science.

The retreat followed the format of the previous ones, intended to give the fullest opportunities for students and postdoctoral researchers to present their research and seek collaborations. It featured three keynote lectures from external distinguished scientists, shorter oral presentations from students and postdocs, and an extensive poster session. This year, in a development funded by the Roberts Fund for innovation in training for early-stage researchers, it also included a fun and informative session tantalizingly described in the programme as "research speed dating".

The meeting opened with a short welcome address given by the ISMB director, **Professor Gabriel Waksman**.

Finn Werner (Research Department of Structural and Molecular Biology, UCL) then introduced the first keynote speaker, **Patrick Cramer** from the Gene Center of the University of Munich, Germany. Cramer has made many distinguished contributions to the study of the structures and mechanisms involved in the process of gene transcription in eukaryotes. When he was a postdoc with Roger Kornberg, who shared the 2006 Nobel Prize in Chemistry, Cramer was responsible for the technical breakthroughs in X-ray crystallography that allowed the high resolution structure of the key enzyme RNA polymerase II to be determined.

RNA polymerase is the enzyme that catalyses the synthesis of RNA on its template DNA. Eukaryotes – all multi-cellular organisms and some single-celled ones, including yeasts – have three different forms of this enzyme, each synthesizing one of the three main types of RNA. RNA polymerase II, the smallest of the three with 12 subunits, synthesizes the messenger RNA that forms the template for protein synthesis. The Cramer lab has used X-ray crystallography to solve structures of this protein complex bound to its different DNA substrates and a growing DNA-RNA double helix. These structures are precise enough for the atomic-level interactions between amino acids and nucleic acids in the protein active site cleft to be discerned. Many stages of the molecular process of adding a new nucleotide to RNA have been "frozen" in space and time by the Cramer, Kornberg, and Vassylyev laboratories, allowing movies to be produced showing the conformational changes involved. Further structural studies are elucidating the molecular mechanisms of processes such as the maturation of raw messenger RNA into the form that is used as a template for protein synthesis, and the repair of RNA that contains mismatched bases.

Dr. Werner then introduced the first session of student and postdoc presentations. **Qing Cai,** who works with Dr Andrea Townsend-Nicholson in the Research Department of Structural and Molecular Biology at UCL spoke first, describing the identification and characterization of a dimer of G-protein coupled receptors, the A_1:$P2Y_1$ heterodimer. The A_1 receptor binds adenosine, and the $P2Y_1$ receptor binds the nucleotides ADP and ATP; these two receptors are known to dimerise together in mammalian brain tissue. Using fluorescence assays among others, Cai has demonstrated that constitutive activity at the A_1 receptor is required for a response to nucleotides, and that the activity of the receptor dimer is coupled to the calcium signaling pathway.

The second presentation was given by **Dimitrios Evangelopoulos**, a PhD student with Dr Sanjib Bhakta in the School of Biological and Chemical Sciences at Birkbeck. He discussed a novel biochemical pathway in mycobacteria. The most notorious member of this genus is *Mycobacterium tuberculosis*, the causative agent of tuberculosis. Strains of tuberculosis that are resistant to many of the main drugs used to treat this disease – so-called extremely drug-resistant tuberculosis (XDR-TB) are becoming a global health emergency and there is an urgent need to develop novel anti-tubercular drugs. Evangelopoulos' work focuses on the characterization of the gene for arylamine N-acetyltransferase (NAT), which is found in many mycobacterial species and which is involved in developing resistance to one important anti-tubercular drug, isonazid. Mutant bacteria with deletions of this gene are being used to investigate the metabolic pathways that it is involved in.

The second session of student and postdoc presentations was chaired by **Carolyn Moores** from the School of Crystallography at Birkbeck. **James Galman** from Helen Hailes' research group in UCL's Department of Chemistry gave the first talk, which was entitled "Adventures with Transketolase". This enzyme, which may be isolated from yeast or from spinach leaves, catalyses the transfer of a two-carbon chemical group termed a ketose from one molecule to another. This process can be important synthetically, in the industrial production of common drug molecules including steroids and some antibiotics. Galman has used a technique called iterative saturation mutagenesis (ISM) to generate a comprehensive set of active-site mutants of this enzyme, and is now using these to investigate the substrate scope of the enzyme. There is considerable industrial interest in novel ketodiol molecules that can be synthesized, catalysed by these mutant enzymes.

Eddy Goh from the Research Department of Structural and Molecular Biology at UCL gave a talk entitled "A nuclear complex of S6K2, mTOR and hnRNP F drives cell proliferation". Both S6K2 (S6 kinase 2) and mTOR (mammalian target of rapamycin) are kinases involved in the regulation of cell growth and cell size; mutant organisms with the S6K2 gene deleted are viable but small in size. Both proteins are part of the same metabolic network, which is regulated by nutrients and growth factors, and mTOR is a regulator of S6K2. Goh is investigating the pathways through which they regulate and drive cell proliferation.

Newts – and other urodele amphibians – have, unique among vertebrates, the ability to regenerate lost limbs. **Kathrin Grassme**, also from the Research Department of Structural and Molecular Biology, presented some insights into the biochemical nature of this process. The newt Anterior Gradient (nAG) protein is a growth factor that is expressed in nerve sheath and mediates limb regeneration. It is a member of the thioredoxin super-family, with a typical thioredoxin fold, but unlike most thioredoxins it contains only one cysteine residue in its catalytic site. Grassme has shown, using homology modeling and site-directed mutagenesis, that this cysteine is necessary for its biological activity.

The first day's science finished with the "research speed dating" session. This – similar to its much better known romantic counterpart – was a formalized method for meeting new people and discussing topics of (generally scientific) mutual interest. Structural biologists and bioinformaticians sat at tables, and researchers from other disciplines – mostly molecular and chemical biology – moved from one to another in a set order, with six minutes allotted for each interaction. Many researchers

were initially skeptical about how this would work, but most seemed to find it a rewarding experience in practice. A wide variety of successful encounters took place, although most of these will not result in new full research collaborations; some "dates" simply exchanged tips about research methods or teaching techniques. "I was originally dubious about how it would work, but I would now be keen on doing it again", commented one of the organizers, Snezana Djordjevic (Research Department of Structural and Molecular Biology, UCL).

If there was a general complaint about the speed dating, it was that the session was rather on the long side; talking non-stop for over ninety minutes proved exhausting. It was possibly just as well that it was followed by an excellent dinner. And after dinner, delegates continued to mingle in the poster session. Forty-nine posters were presented, covering a wide range of topics within the disciplines of structural and molecular biology, biological chemistry and bioinformatics and representing research from all four life science-based departments within the ISMB.

Attendees at the poster session during the evening of Thursday 24 June

The second day started with a fascinating keynote talk by **Professor Christopher Schofield** of the Department of Chemistry at the University of Oxford, UK. **Derek Macmillan** (UCL Department of Chemistry), who chaired the session, praised Schofield's broad range of research interests, describing him as "world-leading in each one". Schofield described the process of oxygen sensing in mammals. This story can be traced back to the first observations in the late nineteenth century of an increase in the number of erythrocytes (red blood cells) in climbers at high altitude, where oxygen concentration is lower. However, the molecular mechanisms involved in sensing and responding to low oxygen concentration – they hypoxic response – are only now being elucidated in any detail.

At the molecular level, two separate hypoxic responses can be distinguished: an acute response, which takes seconds and is modulated by ion channel activity, and a long-term response, which can last for weeks to years and is induced by protein synthesis. The first real breakthrough in understanding the second of these mechanisms came with the discovery of the transcription factor complex Hypoxia Inducible Factor 1 (HIF-1), which regulates the production of a protein, erythropoietin, which is involved in the production of red blood cells. This is a hetero-dimer, but the level of only one of the two subunits – the alpha subunit – varies with oxygen concentration. HIF-1α contains three domains, involved, respectively, in DNA binding and dimerisation; degradation; and transactivation. The central domain contains two proline residues which can be oxidized by HIF prolyl hydrolase (PHD) to form hydroxyproline; the presence of hydroxyproline makes HIF-1α a target for rapid degradation by the proteasome. HIF-1α, therefore, is only stable under low oxygen conditions; mutating either proline residue to alanine gives a constitutively stable form. The hydroxylase that catalyses this reaction binds oxygen to iron (II) at its active site unusually tightly, and reacts with it unusually slowly; this slow reaction, which is conserved in all animals, is consistent with its role as an oxygen sensor. Schofield finally discussed the prospects for therapeutic intervention in this pathway, perhaps stimulating PHD activity to reduce angiogenesis or reducing it to treat anaemia. Most pharmaceutical companies now have at least one early-stage project targeting the HIF hydroxylase pathway.

This lecture was followed by a further series of presentations by students and postdoctoral researchers from Birkbeck and UCL. **Angela Hirtreiter** from the Department of Biochemistry at UCL described her studies of the process of transcriptional elongation by archaeal RNA polymerases. During transcription, a number of proteins termed elongation factors are required to maintain the momentum of RNA synthesis. These have differing mechanisms, some binding to the DNA or RNA and others interacting with the RNA polymerase enzyme. The RNA polymerase subunits F and E play an important role in both transcription initiation and elongation. Hirtreiter and her colleagues are identifying the molecular role of these subunits;

they have determined that these subunits both bind RNA and regulate the so-called RNA polymerase "clamp", and that their role in elongation is stimulated by elongation factors known as Spt4/5, which are conserved in all domains of life.

Hugh Martin, who works with Peter Coveney in the Chemistry Department at UCL, described a fundamental biochemical process with important implications for biotechnology as well as medicine. Polynucleotides often need to translocate across cell membranes through pores composed of proteins such as alpha-hemolysin. This process can be measured by recording the tiny current that flows across the membrane. Different polynucleotides have different translocation times, and a poly-adenine may take up to twenty times as long to cross the same membrane as poly-deoxycytosine. Martin has used molecular dynamics simulation to determine the free energy profile of these translocations, observing that an increased resistance to translocation for poly-adenine in simulation is very likely to be due to stronger interactions between its phosphate groups and lysine residues in the protein pore. Once this process has been fully understood it may be possible to harness it as a novel gene sequencing technique on the nano scale.

Martin's talk was followed by two describing the structures and mechanisms of bacterial proteins, both speakers coming from the School of Crystallography at Birkbeck. **Claire Naylor**, a postdoc in David Moss' structural biology group, continued the pore theme with a discussion of pore-forming toxins from the bacterium *Clostridium perfringens*. This bacterium can express a number of toxic proteins that are responsible for human and animal diseases ranging from gas gangrene to simple food poisoning. Beta pore-forming toxins are soluble as monomers but undergo conformational changes and oligomerise when bound to cell membranes to form pores. Many bacteria contain these toxins, but those of *C. perfringens* are particularly potent because they bind only to receptors on specific cell types. Naylor and her colleagues in the Moss group have obtained the structures of a number of these toxins, including enterotoxin, and are investigating their mechanism of action. Enterotoxin – the second largest cause of hospital-acquired sickness and diarrhea – is now thought to form its pore through a novel mechanism and only in the presence of the receptor, claudin.

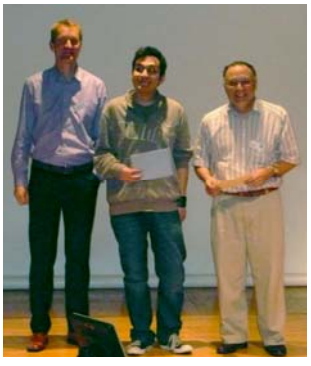
Ana Toste Rêgo, a graduate student with Professor Gabriel Waksman, described the structure of a protein involved in the formation of the hairlike appendages or pili found on the cell surfaces of many bacteria. In gram negative bacteria, the outer membrane "usher" protein PapC is the platform on which pilus subunits assemble and are translocated across the membrane. It was the last major pilus protein for which atomic-level structural information was determined, and even now its C-terminal domain, known to be necessary for pilus assembly, is not well characterized. Toste Rêgo described this as "the last piece of the puzzle". She has cloned and expressed a number of C-terminal regions of the usher protein, and selected one stable small domain for further studies. In collaboration with Professor Sheena Radford of Leeds University, she is using electrospray ionization mass spectrometry (ESI-MS) to show its interaction with the chaperone-subunit complexes, such as the pilus proteins PapD - PapG (chaperone-adhesin) complex, and is aiming to solve its structure using NMR in collaboration with Professor Paul Driscoll at UCL.

The final speaker was **Oliver Willhoft**, also from Birkbeck's School of Crystallography. Willhoft, a Ph.D. student working with Dr Cara Vaughan, is studying a protein complex that binds to the DNA at chromosome centromeres and is involved in the formation of the kinetochore, a much larger protein-nucleic acid complex that links the chromosome to the microtubules during mitosis. This initial complex – known as CBF3 – can be thought of as the "anchor" of the kinetochore. It comprises four proteins, Ndc10, Cep3, Skp1 and Ctf13, and requires the chaperone Hsp90 and its co-chaperone Sgt1 to bind DNA. Willhoft and Vaughan are unraveling the process of CBF3 formation by adding proteins sequentially to the chaperone - co-chaperone complex, and have shown that a subcomplex between Sgt1 and Skp1 forms in the presence of Hsp90 only. They are currently evaluating the role of Cft13, which is unstable on its own.

The scientific proceedings concluded with a final keynote lecture from **Jan Löwe**. Löwe had traveled only a few miles, from the MRC Laboratory of Molecular Biology in Cambridge. Introducing him, the session chair, **Caroline Moores** (Birkbeck School of Crystallography) explained that he had done some of the first structural analyses of the bacterial cytoskeleton: a protein structure that is found within cells, that maintains their shape and is involved in many transport mechanisms. His talk, on plasmid segregation by the protein complex ParMRC, told part of the "prokaryotic cytoskeleton story". Prokaryotic cells are much simpler than eukaryotic ones and were originally thought to contain no cytoskeleton at all. However, prokaryotic proteins with similar functions to the eukaryotic cytoskeletal proteins actin and tubulin are now being discovered and characterized. The first to be discovered was FtsZ, a ring-shaped protein that is important in bacterial cell division. It has weak sequence similarity to tubulin and is now thought to be evolutionarily related to it. Another protein, MreB, is known to control cell shape and to be similarly related to actin.

Löwe, however, focused on filamentous proteins that are encoded on plasmids and that are involved in the segregation of plasmid DNA inside cells, in "the simplest mitotic machinery that we know of". ParM, which is structurally similar to the actin-like protein MreB, forms double-helical filaments that can extend from one end of a cell to the other. It interacts with ParR and ParC to segregate plasmids when the bacteria divide. ParR is a small DNA-binding protein which forms a dimer and is structurally similar to some repressor proteins. DNA was modeled, and then found experimentally using electron microscopy, to bind round this dimer forming a ring-shaped structure, which in turn can bind to the ends of the ParM filaments. These filaments have been located in mutant and wild-type cells using electron tomography. Wild-type bacteria usually contain only a small number of filaments, close to the usual plasmid copy number of five. They cluster near to the nucleoid, the region in the bacterial cell where the nuclear material, including the plasmids, is located. Divided plasmids are now thought to bind to ParM during mitosis via these ring-shaped complexes, and to be pulled apart by its elongation.

The final event of the meeting was a very pleasant one: the award of the prizes for the best poster and oral presentations. **Angela Hirtreiter** won the presentation prize for her talk, "Transcriptional elongation by archaeal RNA polymerase". The poster prize was won by **Ricardo Aramayo**, a postdoctoral researcher working in Professor Elena Orlova's electron microscopy group in the School of Crystallography, Birkbeck. Aramayo's very attractive poster described an elegant structural study of the tumour suppressor protein p53 bound to DNA. This is the first EM structure of the intact protein-DNA complex, and it reveals the DNA bound to one side of the p53 tetramer.



Ricardo Aramayo, winner of the "ISMB Young Investigator Award for best poster", with Jan Löwe on the left and Gabriel Waksman on the right

Angela Hirtreiter, winner of the "ISMB Young Investigator Award for best talk", with Jan Löwe on the left and Gabriel Waksman on the right

Professor Waksman summed up two sessions of excellent science by saying that all the younger presenters had done "incredibly well" and recommended them to aspire to the level of the "exciting" science presented by the keynote speakers. He further praised the skills of the dedicated committee and of the ISMB's administrator, Anne-Cécile Maffat. We are already looking forward to a similar rich scientific experience at the fourth ISMB retreat in 2011.

Pictures: S.Perkins, I.Tsaneva, Robinson College